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Short communication

Development and evaluation of bee venom topical formulation for efficient treatment of arthritis

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Introduction

Arthritis is a chronic, complex autoimmune disease that affects approximately 1% of the global population. Conventional therapeutic management involves usage of steroids, nonsteroidal anti-inflammatory, disease modifying antirheumatic and immunosuppressant drugs. Despite the increasing number of new drugs and treatment regimes, complete long-term disease remission is not achieved for many patients and thus new therapeutic options are required (Guo et al., 2018).

Bee venom (BV) therapy has been used since ancient times. According to animal experiments, BV exhibits antiarthritic, anti-inflammatory and analgesic effects attributable to the suppression of cyclo-oxygenase-2 and phospholipase A2 expression and a decrease in the levels of TNF-α, IL-1 and IL-6, nitric oxide and oxygen-reactive species. Bioactive BV compounds, such as peptides (melittin, adolapin and apamin), enzymes (phospholipase A2) and amines are also associated with these actions (Lee et al., 2014).

The topical delivery is an attractive method for local treatment of inflammatory conditions like musculoskeletal disorders. Topical delivery has many advantages over the conventional oral dosage forms, especially in avoidance of various adverse effects. Having in mind that the therapeutic efficacy of a topical formulation depends on both the nature of the vehicle and the physicochemical properties of the active agent (release rate, rate and extent of drug permeation, etc.) (Özcan et al., 2009), the aim of this study was to develop an effective, stable topical gel formulation containing BV as an active agent.

Materials and methods

BV was collected by electric stunning, without harming honey bees during July 2019 (Kozarac, BiH) and was stored at -20 °C. A modified HPLC method (Rybak-Chmielewska and Szczêsna, 2004) was used for assay of BV using melittin (Sigma, USA) as an external standard (Agilent Technologies 1200 Series; Restek Ultra C18 column; gradient elution with 0.1% trifluoroacetic acid (TFA) in water (mobile phase A) and 0.1% TFA in acetonitrile-water 80:20 (mobile phase B), flow rate 2.5 mL/min, 20 µL injection volume, λ of 220 nm).

The gels were prepared by dissolving different concentrations of chitosan (CTS, low-molecular weight; Sigma–Aldrich, USA) in 1% of lactic acid

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solution with or without poloxamer 407 (PL; Pluronic F127, BASF Chemtrade GmbH, Germany) (1.75% CTS+0.5% PL-*sample 1,* 2% CTS+0.5% PL-*sample 2,* 1.75% CTS-*sample 1O,* 2% CTS - *sample 2O,* respectively), 5% of propylene glycol (Alkaloid, N. Macedonia), 0.2% of potassium sorbate (PS; Apac Chem. Corp., USA) and 0.3% of BV by agitatiation at 300 rpm with the aid of magnetic mechanical stirrer (Variomag, Germany).

Prepared gels were characterized for pH (Sartorius, Germany), viscosity (at 25 °C; DV2T, Spindle T bar T-A 91, Brookfield Eng. Lab., Inc., USA) and spreadability (0.5 g of gel covered with 68 g glass plate, during 5 min). PS content was determined UV spectrophotometricaly (264 nm; Lambda 16, Perkin Elmer, USA) after dissolving of 1 g of gel in 10 mL of methanol. *In vitro* BV release from the gels (1.5g) was performed using membrane diffusion cells (MEMBRA-CELL dialysis tubing; Serva Feinbiochemica GmbH, Germany) (32±0.5˚C, 15 mL of distilled water as a dissolution medium, 300 rpm). At predetermined time intervals aliquots were taken and analyzed by HPLC.

Results and discussion

BV assay and quality were determined based on melittin content. Obtained BV sample contained 43.54% of melittin. Prepared gels were characterized with the pH of 4.5-5.2. Having in mind that the consistency is one of the most important features for analgesic and anti-inflammatory topical forms, the gel viscosity plays an important role in drug permeation control. Results from the viscosity measurements showed that by increasing the CTS concentration, the viscosity of the samples increased (5920 cP for *samples 1O* and 11160 cP for *sample 2O*, respectively). By incorporation of 0.5% PL, the viscosity of the gels also increased, probably due to PL properties for micellization and sorting the polymer chains into a denser network (6400 cP for *sample 1* and 18120 cP for *sample 2*, respectively). All prepared samples showed pseudoplastic behavior. The therapeutic efficacy of gels also depends on their spreadability. The proper spreading helps in the uniform application of the gel to the skin and satisfies the ideal quality for topical application. Spreadability factor calculations demonstrated that *sample 2O* had the significantly higher spreadability in comparison to *sample 1O* (p<0.05). By incorporation of PL, both formulations (*sample 1* and *sample 2*) showed similar spreadability as *sample 2O*. The content of potassium sorbate in formulated gels was 99.85±2.04%. The concentration of gelling agents (CTS or CTS/PL combinations) significantly influenced the *in vitro* release behavior of BV from the prepared formulations. Gel formulation prepared with 1.75% CTS and 0.5% PL (*sample 1)* showed highest release rate (~98%) during the period of 24 h, following the Peppas-Sahlin kinetic model.

Conclusion

According to the results obtained from this study it could be concluded that BV was successfully incorporated into the CTS/PL gels formulations. Prepared gels showed suitable pH value, viscosity and spreadability. Formulation prepared with 1.75% CTS and 0.5% PL could be a promising candidate for efficient topical delivery/treatment of arthritis. Further clinical studies should be conducted.

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